

# Identification of starch in milk protein gels using confocal microscopy

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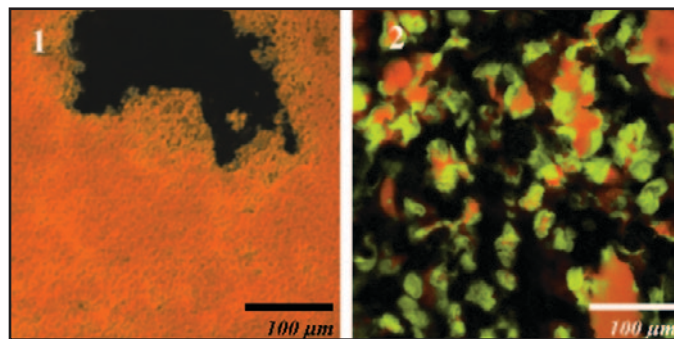


## Introduction

Various starches are common in dairy products. Historically, the standard staining technique for starch has been Lugol's Iodine. This dye, a general stain for starch is not fluorescent and only limited information can be obtained using transmission light. Fluorophores do not attach to starches. We have developed a method in which starches are derivatized so that fluorophores attach.

## Materials and Methods

Milk gels were produced containing a variety of starches including a control gel containing no starch (NS), a modified waxy cornstarch (WC), an instant modified cornstarch (IC), waxy rice starch (RS), instant tapioca starch (ITS), and dextrin (D). Samples were fixed in 1.0% osmium tetroxide in milk serum buffer using a Pelco 3470 microwave system for 20 min at power 2, soaked in 0.5% periodic acid (aq), stained in 1.0% Acriflavin HCl (aq), stained in 0.01% Rhodamine B (aq), rinsing in ddH<sub>2</sub>O three times after each step. Samples were

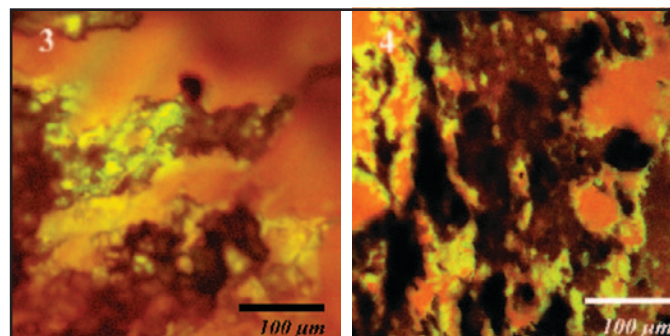


**Figure 1.** Control milk gel with no starch added. Note the even protein fluorescence, and lack of starch fluorescence. **Figure 2.** Milk gel with modified waxy cornstarch (WC) added. Note the large irregular WC granules associated with the milk protein.

mounted on standard microscope slides with glycerin jelly; imaged on a BioRad MRC 23 with a Kr/Ar laser exciting the Acriflavin HCl at 488 nm and the Rhodamine B at 568 nm. Emissions were from 488 to 650 nm and 550 to 750 nm, and exclusion filters of 512 to 532 nm and below 585 nm used to capture the fluorescent signals, respectively. Images were false colored with protein as red-orange and starch as yellow-green.

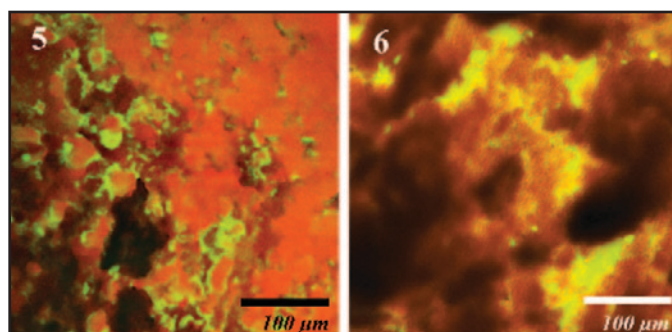
## Results

All of the starches were derivatized and fluoresced. Different starches interacted differently with the milk protein.



**Figure 3.** Milk gel instant modified cornstarch (IC) added. Note the diffuse IC particles associated with the milk protein. **Figure 4.** Milk gel waxy rice starch (RS) added. Note the small regular RS granules associated with the milk protein.

Control images (NS) had no cross-reaction of the protein with Acriflavin HCl, fig. 1. WC had large starch granules associated with the protein, fig. 2. IC had diffuse starch throughout the protein, fig. 3. RC had small regular starch granules associated with the protein, fig. 4. ITS had a non-particulate nature, as it is intermingled within the milk protein, fig. 5. D had very diffuse characteristic throughout the protein, fig. 6. Bright yellow-green particles are believed to be non-gelatinized starch.



**Figure 5.** Milk gel instant tapioca starch (ITS) added. Note the non-particulate nature of the ITS starch as it is intermingled within the milk protein. **Figure 6.** Milk gel dextrin (D) added. Note the totally diffuse nature of the D starch as it is evenly distributed throughout the milk protein.

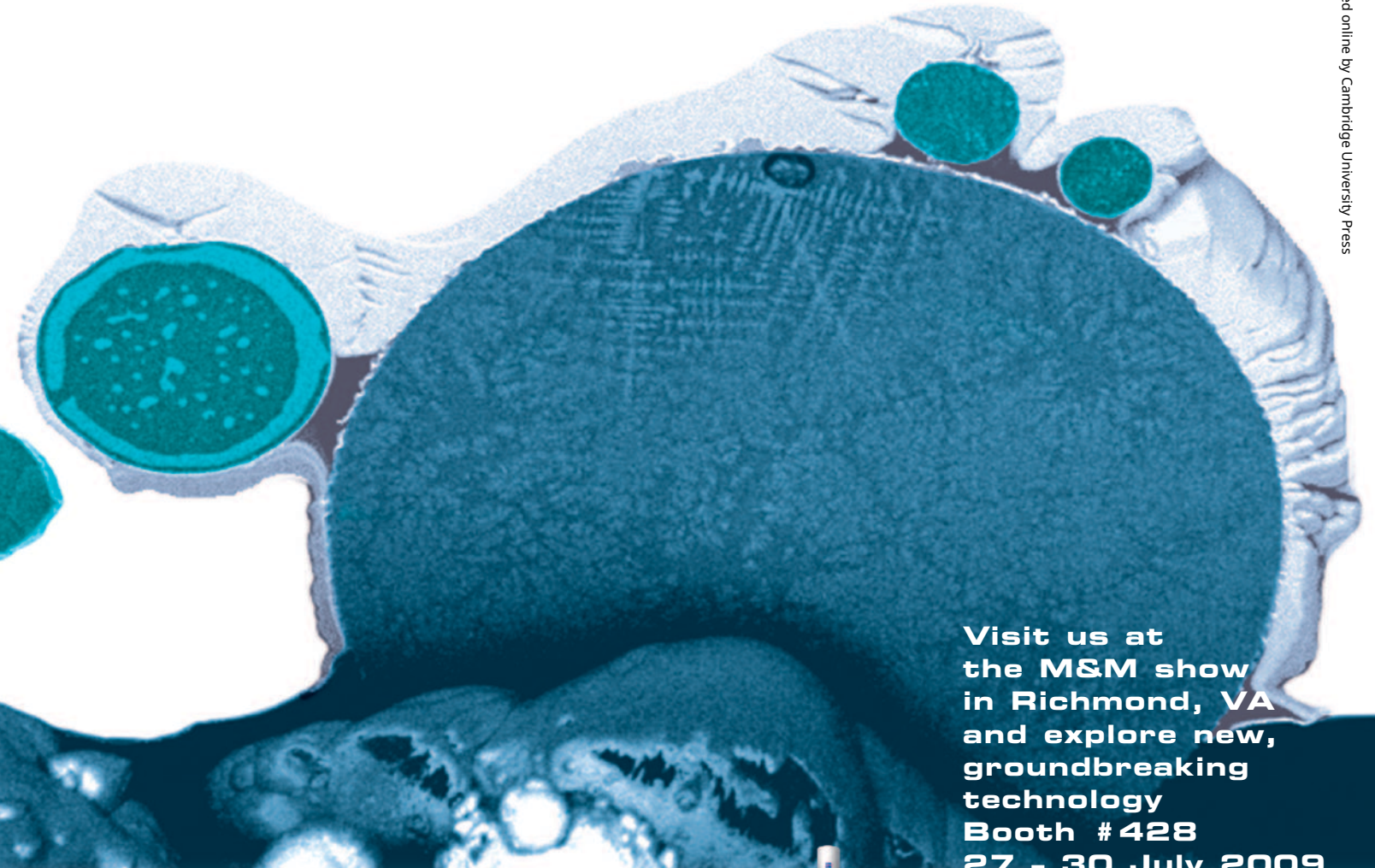
## Conclusions

Periodic acid reacts at the reducing-sugar anomeric carbon site of pentoses and hexoses converting it to dialdehydes, which can then react with the Acriflavin HCl thus forming the fluorescently labeled starches. All of the starches tested fluoresced with this procedure and the varying distributions and structures of the starches were observable. [MT](#)

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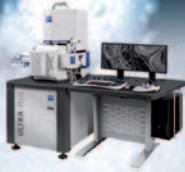
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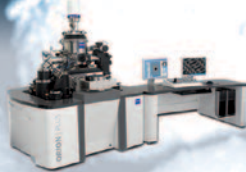
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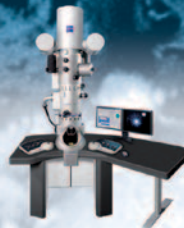
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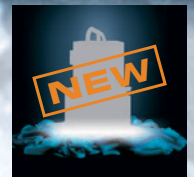
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